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Further Checkpoints in Th1 Development

Minireview

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Tight control of Th1 immunity is essential to prevent immunopathology. Central to control of the IFN-γ gene is the transcription factor T-bet, whose induction is Stat-1 dependent. IL-12 is dominant in directing Th1 development, while synergizing with IL-18 for IFNγ production from differentiated Th1 cells. In this issue of *Immunity*, IL-27 is described, which acts in synergy with IL-12 early in Th1 development from naive T cells via the receptor TCCR/WSX-1. We review the coordination of these checkpoints in Th1 development and function.

Appropriate induction of a Th1 response is required for effective eradication of intracellular pathogens and involves macrophage activation and production of complement fixing and opsonizing antibodies (Abbas et al., 1996; O'Garra, 1998; Murphy et al., 2000). This activity requires optimal IFNγ production from Th1 cells. Initial studies showed that IFNγ itself was required but not sufficient for the development of a Th1 response (Abbas et al., 1996; O'Garra, 1998; Murphy et al., 2000). IL-12, produced by monocytes/macrophages and dendritic cells, was shown to be the dominant factor in development of the Th1 phenotype (Trinchieri, 1995; O'Garra, 1998; Murphy et al., 2000). Although required for effective antimicrobial immunity, dysregulated Th1 responses may lead to immunopathology and have been implicated in organ-specific autoimmunity (Powrie and Coffman, 1993; O'Garra, 1998). Perhaps for this reason, Th1 development is tightly regulated and multiple checkpoints in addition to IFNγ and IL-12 have been identified.

In this issue of *Immunity*, Pflanz et al. describe a new heterodimeric cytokine, related to IL-12 and termed IL-27, which acts early together with IL-12 to trigger IFNγ production by naive $CD4^+$ T cells. They also identify IL-27 as the ligand for TCCR/WSX-1 (Chen et al., 2000; Yoshida et al., 2001), a novel member of the class I cytokine receptor family recently shown to be important for Th1 development.

Two groups have shown that mice made deficient in the TCCR/WSX-1 receptor have significant impairment of Th1 responses. Chen et al. showed that upon challenge with protein antigen, TCCR-deficient ($TCCR^{-/-}$) mice had impaired Th1 responses, as measured by IFNγ production, as well as reduced IgG_{2a} production compared to wild-type mice (Chen et al., 2000). Moreover, there was a profound defect in clearance of *Listeria monocytogenes* in these TCCR-deficient mice. An IL-12-driven Th1 response from enriched $CD4^+$ T cells from $TCCR^{-/-}$ mice stimulated in vitro was also significantly impaired as compared to wild-type mice. However, IL-12-induced splenocyte proliferation and upregulation of the IL-12Rβ1 and 2 expression upon Con A activation of splenocytes was unimpaired. More recently, Yoshida et al. showed that $CD4^+$ T cells obtained from $WSX\text{-}1^{-/-}$ mice (an alternative name for TCCR) showed reduced IFNγ production upon primary stimulation under similar Th1-inducing conditions to those used by Chen et al. (2000) (IL-12, anti-IL-4, IL-2, Con A, and irradiated spleen cells) (Yoshida et al., 2001). However, in contrast to the findings of Chen et al., these T cells appeared to recover with respect to IFNγ production upon secondary restimulation in vitro. $WSX\text{-}1^{-/-}$ mice were markedly more susceptible to *Leishmania major* infection, showing increased footpad swelling and increased numbers of organisms recovered from tissues as compared to wild-type C57Bl/6 mice. There was impaired IFNγ production early in the infection (at 2 weeks), although, in accordance with the in vitro data on Th1 development, at 4 weeks after infection, *L. major*-induced IFNγ production by draining lymph node cells restimulated in vitro was equivalent in $WSX\text{-}1^{-/-}$ and wild-type mice. However, this recovery of IFNγ production was not obviously associated with healing of the disease, since footpad swelling and numbers of infiltrating organisms remained elevated as compared to wild-type littermates. This early requirement for effective Th1 immunity in *L. major* clearance was paralleled by increased granuloma formation to BCG. However, in this case hepatic mycobacterial counts were not affected, suggesting that other mechanisms can control some bacterial infections. Thus, both studies indicate that mice deficient in a functional TCCR/WSX-1 receptor show impaired Th1 responses accompanied by an impairment in the clearance of pathogens, including *L. monocytogenes* and *L. major*. However, the latter study suggests that this requirement for signaling through TCCR/WSX-1 may be most important early in an immune response.

IL-27, described by Pflanz et al. in this issue of *Immunity*, is identified as the ligand for TCCR/WSX-1 (Chen et al., 2000; Yoshida et al., 2001). The authors show that this cytokine is a heterodimer consisting of EBI3, an IL-12 p40-related protein (Devergne et al., 1997), and p28, a newly discovered IL-12 p35-related polypeptide (Pflanz et al., 2002). Furthermore, expression of both components of the heterodimer is upregulated in antigen-presenting cells upon activation with LPS. IL-27 induced significant proliferation of naive but not memory T cells (both in mouse and human systems) in the absence of IL-2. These findings are in keeping with a higher level of mRNA expression for TCCR by undifferentiated

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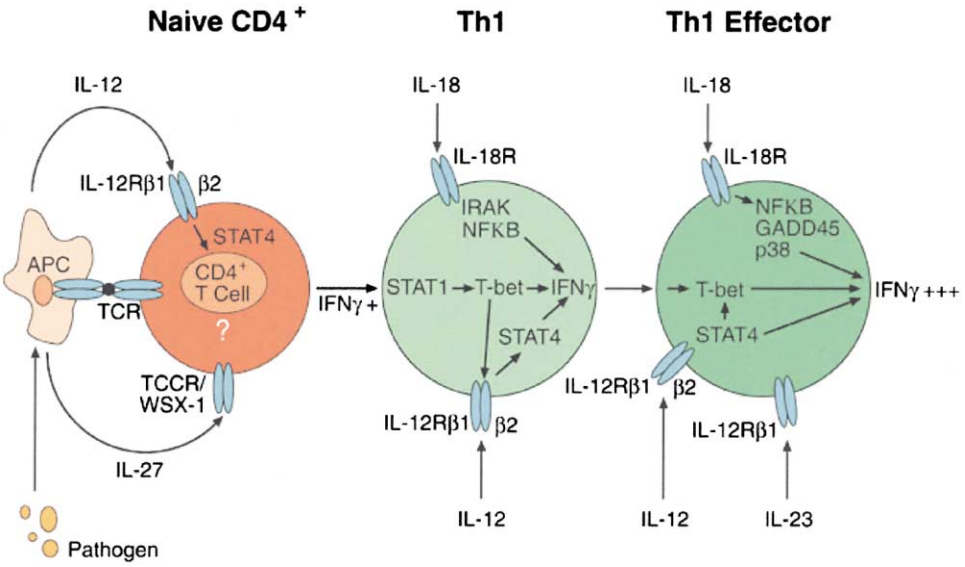

Figure 1. Checkpoints in Th1 Development

Presentation of pathogen antigens to naive T cells by antigen-presenting cells (APC) induces IL-12Rβ2 expression on T cells. APC-derived IL-12, together with IL-27, initiates Th1 development. IL-27 signals through the T cell cytokine receptor (TCCR, also termed WSX-1). IL-12 acts via Stat4 to upregulate IFNγ production. Th1 development requires IFNγ itself, which activates Stat1 and induces expression of the transcription factor T-bet. T-bet is a major Th1 commitment factor and transactivates the IFN-γ gene, as well as inducing chromatin remodeling of the IFN-γ locus. IL-12 induces IL-18 receptor expression, which allows IL-18 to synergize with IL-12 to increase IFNγ production from committed Th1 effectors. IL-18 signals via IL-1 receptor-associated kinase (IRAK) to activate NFκB, and the combination of IL-12 and IL-18 activates GADD45 and p38 mitogen kinase signaling, all of which are major amplifiers of the IFNγ response. Memory $CD4^+$ T cells respond to IL-23, an IL-12-related cytokine, which increases proliferation and may increase IFNγ production further.

$CD4^+$ T cells as compared to Th1 or Th2-polarized cells (Chen et al., 2000). It had previously been shown that IL-12Rβ2 was not expressed by naive $CD4^+$ T cells but was induced upon activation through the TCR, and its expression was increased during IL-12-induced Th1 development (Szabo et al., 1997; Rogge et al., 1997). Since IL-27 synergized with IL-12 for induction of IFNγ production by naive T cells, this suggests that IL-27 may prime T cells for subsequent IL-12-induction of IFNγ, or, conversely, that both signals are required. However, Chen et al. (2000) have shown that the TCCR/WSX-1 receptor (for IL-27) was not required for the induction of the IL-12R on splenocytes upon activation. Thus, IL-27 appears to act at an early stage in Th1 development in a manner distinct from IL-12.

The biology of IL-27 distinguishes it from the earlier described cofactor for IL-12-driven Th1 development, IL-18, which appears to act later in Th1 development (Okamura et al., 1995; Robinson et al., 1997). IL-18 is an IL-1 family member which, as well as enhancing IL-12-driven Th1 development, shows pronounced synergy with IL-12 for secretion of IFNγ by differentiated Th1 cells (Robinson et al., 1997). Interestingly, IL-18 with IL-12 can induce IFNγ production from Th1 cells in the absence of T cell receptor signaling (Robinson et al., 1997; Yang et al., 2001; Lertmemongkolchai et al., 2001), thus augmenting antigen-specific Th1 immunity. The receptor for IL-18 (an IL-1R family member) is induced by IL-12 (Afkarian et al., 2002).

IL-12 signals through Stat4 activation for the development of Th1 responses (Szabo et al., 1997; Jacobson et al., 1990; Kaplan et al., 1996), and loss of the IL-12Rβ2 and thus IL-12-induced Stat4 activation was postulated to be a commitment step to explain why Th2 cells cannot produce IFNγ (Szabo et al., 1997; Rogge et al., 1997). However, recently it was shown that ectopic expression of the IL-12Rβ2 in developing Th2 cells restored IL-12-induced Stat4 activation and proliferation but not IFNγ production (Heath et al., 2000; Nishikomori et al., 2000). This suggests additional pathways for IFNγ induction.

The transcription factor T-bet has recently shown to have an important role in Th1 development. T-bet was isolated from yeast two-hybrid analysis of binding of cDNAs from an activated Th1 library to a Th1-specific portion of the IL-2 promoter, coupled with RDA (Szabo et al., 2000). T-bet expression correlated with IFNγ production by Th1 cells and NK cells, and T-bet was a potent transactivator of the IFNγ gene. Retroviral gene transduction of T-bet into primary T cells or developing Th2 cells could activate IFNγ production (Szabo et al., 2000). T-bet-deficient mice had default Th2 development and developed spontaneous airway changes similar to those of asthma (a Th2-driven disease) (Finotto et al., 2002), although it is of note that T-bet did not appear to be required for IFNγ production by $CD8^+$ T cells (Szabo et al., 2002). T-bet induction and Th1 development could still occur in Stat4-deficient mice (Mullen et al., 2001) when T cells were stimulated in the presence of anti-IL-4. Furthermore, ectopic expression of T-bet by a retroviral vector induced IFNγ production from $Stat4^{-/-}$ T cells even when cultured in IL-4 and anti-IL-12. T-bet thus appears to act before and independently of IL-12-induced Stat4 activation in Th1 development and induces chromatin remodeling of the IFNγ locus.

However, other investigators reported that Stat4 activation could further increase T-bet expression (Grogan et al., 2001). More recently, two groups have shown that T-bet expression is induced in a Stat1-dependent manner via IFNγ (Lighvani et al., 2001, Afkarian et al., 2002). Indeed, Stat1-deficient T cells failed to express T-bet despite IFNγ induction under Th1-polarizing conditions (IL-12 and anti-IL-4) (Afkarian et al., 2002). These investigators also showed that T-bet induced IL-12Rβ2 expression and could do so independently of Stat1 but found no evidence for autoactivation of T-bet (Afkarian et al., 2002). Thus, one reason for failure of T-bet-deficient T cells to mount Th1 responses could be an inability to induce the IL-12Rβ2. However, expression of the IL-12Rβ2 was seen in Th1 cells derived from Stat1$^{-/-}$ mice (Afkarian et al., 2002), which may indicate a T-bet-independent pathway to IL-12Rβ2 expression that could also be via IL-27. It will be of interest to see if this is the case, and also whether IL-27 induces Stat1 activation or T-bet expression. The suggestion from the TCCR$^{-/-}$ mice is, however, that IL-12R expression does not require IL-27 signaling. Thus, any role of IL-27 in inducing IL-12 responsiveness remains speculative (Chen et al., 2000).

A number of other signaling pathways have been implicated as potential checkpoints in Th1 development and IFNγ production, including IL-18 signaling via NFκB (Robinson et al., 1997), p38 MAPK (Lu et al., 2001), and GADD45 (Yang et al., 2001). Another IL-12-related cytokine, IL-23, consisting of a novel protein, p19, which binds to the p40 subunit of IL-12, has been reported to act selectively in proliferation and IFNγ production from memory T cells (Oppmann, et al., 2000).

The definition of the IL-27 interaction with TCCR/WSX-1 is a further step in understanding the complex checkpoints in Th1 development and underpins the importance of multiple levels of control for IFNγ production for achieving optimal Th1 protective immunity to infection without induction of immunopathology. Perhaps most important for protection from intracellular pathogens are IL-12 and IL-18, which synergistically induce maximal IFNγ production from Th1 cells (Neighbors et al., 2001). These checkpoints in Th1 development are summarized in Figure 1.

It is of note that dramatic clinical syndromes result from defects in the Th1 regulation pathway, including IFNγR, IL-12R, and Stat1 deficiency, which cause profound immunodeficiency (Jouanguy et al., 1996; Ottenhoff et al., 1998; Dupuis et al., 2001). It will be of interest to see whether similar syndromes occur for T-bet, IL-27, or TCCR/WSX-1 and whether specific targeting of these Th1 checkpoints will be useful for manipulating immunity to infection or ameliorating Th1-induced autoimmune pathology.

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